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(57) Abstract

Novel burst-free, sustained release biocompatible and biodegradable microcapsules which can be programmed to release their active core for variable durations ranging from 1-100 days in an aqueous physiological environment. The microcapsules are comprised of a core of polypeptide or other biologically active agent encapsulated in a matrix of poly(lactide/glycolide) copolymer, which may contain a pharmaceutically acceptable adjuvant, as a blend of upcapped free carboxyl end group and end-capped forms ranging in ratios from 100/0 to 1/99.

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THERAPEUTIC TREATMENT AND PREVENTION OF INFECTIONS WITH A BIOACTIVE MATERIALS ENCAPSULATED WITHIN A BIODEGRADABLE-BIOCOMPATIBLE POLYMERIC MATRIX

I. GOVERNMENT INTEREST

The invention described herein may be manufactured, used and licensed by or for the Government for Govermental purposes without the payment to use of any royalties thereon.

II. CROSS REFERENCE

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/590,973 filed January 24, 1996 which in turn is a continuation-in-part of U.S. Patent Application Serial No. 08/446,149 filed May 22, 1995. which in turn is a continuation of U.S. Patent Application Serial No. 590,308 dated March 16, 1984.

Additionally, this application is a continuation-in-part of U.S. Patent Application Serial No. 08/446,148 filed May 22, 1995, which in turn is a continuation-in-part of U.S. Patent Application Serial No. 08/867,301 filed April 10, 1992 now U.S. Patent No. 5,417,986 issued May 23, 1995, which in turn is a continuation-in-part of U.S. Patent Application Serial No. 590,308 filed March 16, 1984.

III. FIELD OF THE INVENTION

This invention relates to compositions comprising active core material(s) such as biologically active agent(s), drug(s) or substance(s) encapsulated within an end-capped or a blend of uncapped and end-capped biodegradable-biocompatable poly(lactide/glycolide) polymeric matrix useful for the effective prevention or treatment of bacterial, viral, fungal, or parasitic infections, and combinations thereof. In the areas of general and orthopedic surgery, and the treatment of patients with infectious or chronic disease conditions, this invention will be especially useful to physicians, dentists and veternarians.

IV. BACKGROUND OF THE INVENTION

Wounds characterized by the presence of infection, devitalized tissue, and foreign-body contaminants have high infection rates and are difficult to treat.

To prevent infection, in bone and soft tissue systemic antibiotics must be administered within 4 hours after wounding when circulation is optimal. This has been discussed by J.F. Burke in the article entitled "The Effective Period of Preventive Antibiotic Action in Experimental Incisions and Dermal Lesions", Surgery, Vol. 50, Page 161 (1961). If treatment of bacterial infections is delayed, a milieu for bacterial growth develops which results in complications associated with established infections. (G. Rodeheaver et al., "Proteolytic Enzymes as Adjuncts to Antibiotic Prophylaxis of Surgical Wounds", American Journal of Surgery, Vol. 127, Page 564 (1974)). Once infections are established it becomes difficult to systemically administer certain antibiotics for extended periods at levels that are safe and effective at the

wound site. Unless administered locally, drugs are distributed throughout the body, and the amount of drug hitting its target is only a small part of the total dose. This ineffective use of the drug is compounded in the trauma patient by hypovolemic shock, which results in a decreased vascular flow to tissues. (L. E. Gelin et al., "Trauma Workshop Report:Schockrheology and Oxygen Transport", Journal Trauma., Vol. 10, Page 1078 (1970)).

Additionally, infections caused by multiple-antibiotic resistant bacterial are on the up-swing and we are on the verge of a potential world-wide medical disaster. According to the Centers for Disease Control, 13,300 patients died in U.S. hospitals in 1992 from infections caused by antibiotic-resistant bacteria. Methicillin-resistant S. aureus (MRSA) is rapidly emerging as the "pathogen of the 90's":

a. Some major teaching hospitals in U.S. report that up to 40% of strains of S. aureus isolated from patients are resistant to methicillin. Many of these MRSA strains are susceptible only to a single antibiotic (vancomycin).

b. Should MRSA also develop resistance to vancomycin, the mortality rate among patients who develop MRSA infections could approach 80%, thereby increasing the threat of this infectious killer.

Moreover, Vancomycin resistance is on the up-swing:

a. 20% of Enterococci are now resistant to vancomycin

b. In 1989, only one hospital in New York City reported vancomycin-resistant Enterococci. By 1991, the number of hospitals reporting vancomycin resistance rose to 38.

c. transfer of vancomycin-resistant gene (via plasmid) has been shown experimentally between Enterococcus and S. aureus.

Many major pharmaceutical companies around the world have either completely eliminated or significantly reduced their research and development programs in the area of antibiotic research. According to a 1994 report by the Rockefeller University Workshop in Multiple Antibiotic Resistant Bacteria, we are on the verge of a "medical disaster that would return physicians back to the pre-penicillin days when even small infections could turn lethal due to the lack of effective drugs."

Despite recent advances in antimicrobial therapy and improved surgical techniques, osteomyelitis (hard tissue or bone infection) is still a source of morbidity often necessitating lengthy hospitalization. The failure of patients with chronic osteomyelitis to respond uniformly to conventional treatment has prompted the search for more effective treatment modalities. Local antibiotic therapy with gentamicin-impregnated poly(methylmethacrylate) (PMMA) bead chains (SEPTOPAL TM, E. Merck, West Germany) has been utilized in Germany for the treatment of osteomyelitis for the past decade and has been reported to be efficacious in several clinical studies. The beads are implanted into the bone at the time of surgical intervention where they provide significantly higher concentrations of gentamicin than could otherwise be achieved via systemic administration. Serum gentamicin levels, on the other hand, remain extremely low thereby significantly reducing the potential for nephro- and ototoxicity that occurs in some patients receiving gentamicin systemically.

Since SEPTOPAL TM is not currently approved by the Food and Drug Administration for use in the United States, some orthopedic surgeons in this country are fabricating their own "physician-made beads" for the treatment of chronic osteomyelitis. A major disadvantage of the beads, however, is that because the PMMA is not biodegradable it represents a foreign body and should be removed at about 2-weeks postimplantation thereby necessitating in some cases an additional surgical procedure. A biodegradable-biocompitable, antibiotic carrier, on the other hand, would eliminate the need for this additional surgical procedure and may potentially reduce both the duration as well as the cost of hospitalization.

The concept of local, sustained release of antibiotics into infected bone is described in recent literature wherein antibiotic-impregnated PMMA macrobeads are used to treat chronic osteomyelitis. The technique as currently used involves mixing gentamicin with methylmethacrylate bone cement and molding the mixture into beads that are 7mm in diameter. These beads are then locally implanted in the infected site at the time of surgical debridement to serve as treatment. There are, however, significant problems with this method. These include: 1) initially, large amounts of antibiotics diffuse from the cement but with time the amount of antibiotic leaving the cement gradually decreases to subtherapeutic levels; 2) the bioactivity of the antibiotic gradually decreases; 3) methylmethacrylate has been shown to decrease the ability of polymorphonuclear leukocytes to phagocytize and kill bacteria; 4) the beads do not biodegrade and usually must be surgically removed; and 5) the exothermic reaction that occurs during curing of methymethacrylate limits the method to

the incorporation of only thermostable antibiotics (primarily aminoglycosides). Nevertheless, preliminary clinical trials using these beads indicate that they are equivalent in efficacy to longer term (4-6 weeks) administration of systemic antibiotics.

In many instances, infectious agents have their first contact with the host at a mucosal surface; therefore, mucosal protective immune mechanisms are of primary importance in preventing these agents from colonizing or penetrating the mucosal surface. Numerous studies have demonstrated that a protective mucosal immune response can best be initiated by introduction of the antigen at the mucosal surface, and parenteral immunization is not an effective method to induce mucosal immunity. Antigen taken up by the gut-associated lymphoid tissue (GALT), primarily by the Peyer's patches in mice, stimulates T helper cell (Th) to assist in IgA B cell responses or stimulates T suppressor cells (Ts) to mediate the unresponsiveness of oral tolerance. Particulate antigen appears to shift the response towards the (Th) whereas soluble antigens favor a response by the (Ts). Although studies have demonstrated that oral immunization does induce an intestinal mucosal immune response, large doses of antigen are usually required to achieve sufficient local concentrations in the Peyer's patches. Unprotected protein antigens may be degraded or may complex with secretory IgA in the intestinal lumen.

In the process of vaccination, medical science uses the body's innate ability to protect itself against invading agents by immunizing the body with antigens that will not cause the disease but will stimulate the formation of antibodies that will protect againts the disease. For example, dead organisms

are injected to protect against bacterial diseases such as typhoid fever and whooping cough, toxins are injected to protect against viral diseases such as poliomyelitis and measles.

It is not always possible, however, to stimulate antibody formation merely by injecting the foreign agent. The vaccine preparation must be immunogenic, that is, it must be able to induce an immune response. Certain agents such as tetanus toxoid are innately immunogenic, and may be administered in vaccines without modification. Other important agents are not immunogenic, however, and must be converted into immunogenic molecules before they can induce an immune response.

The immune response is a complex series of reactions that can generally be described as follows:

1. the antigen enters the body and encounters antigen-presenting cells which process the antigen and retain fragments of the antigen on their surfaces;

2. the antigen fragment retained on the antigen presenting cells are recognized by T cells that provide help to B cells; and

3. the B cells are stimulated to proliferate and divide into antibody forming cells that secrete antibody against the antigen.

Most antigens only elicit antibodies with assistance from the T cells and, hence, are known as T-dependent (TD). These antigens, such as proteins, can be processed by antigen presenting cells and thus activate T cells in the process described above. Examples of such T-dependent antigens are tetanus and diphtheria toxoids.

Some antigens, such as polysaccharides, cannot be properly processed by antigen presenting cells and are not recognized by T cells. These antigens do not require T cell assistance to elicit antibody formation but can activate B cells directly and, hence, are known as T-independent antigens (TI). Such T-independent antigens include H. influenzae type by polyribosyl-ribitol-phosphate and pneumococcal capsular polysaccharides.

T-dependent antigens vary from T-independent antigens in a number of ways. Most notably, the antigens vary in their need for an adjuvant, a compound that will nonspecifically enhance the immune response. The vast majority of soluble T-dependent antigens elicit only low level antibody responses unless they are administered with an adjuvant. It is for this reason that the standard DPT vaccine (diptheria, pertussis, tetanus) is administered with the adjuvant alum. Insolubilization of TD antigens into an aggregated form can also enhance their immunogenicity, even in the absence of an adjuvant. Golub ES and WO Weigle, J. Immunol. 102:389, 1969). In contrast, T-independent antigens can stimulate antibody responses when administered in the absence of an adjuvant, but the response is generally of lower magnitude and shorter duration.

Four other differences between T-independent and T-dependent antigens are:

a) T-dependent antigens can prime an immune response so that a memory response can be elicited upon secondary challenge with the same antigen. Memory or secondary responses are stimulated very rapidly and attain significantly higher titers of antibody that are seen in primary responses.

T-independent antigens are unable to prime the immune system for secondary responsiveness.

b) The affinity of the antibody for antigen increases with time after immunization with T-dependent but not T-independent antigens.

c) T-dependent antigens stimulate an immature or neonatal immune system more effectively than T-independent antigens.

d) T-dependent antigens usually stimulate IgM, IgGl, IgG2a, and IgE antibodies, while T-independent antigens stimulate IgM, IgGl, IgG2b, and IgG3 antibodies.

These characteristics of T-dependent vs. T-independent antigens provide both distinct advantages and disadvantages in their use as effective vaccines. T-dependent antigens can stimulate primary and secondary responses which are long-lived in both adult and in neonatal immune systems, but must frequently be administered with adjuvants. Thus, vaccines have been prepared using only an antigen, such as diphtheria or tetanus toxoid, but such vaccines may require the use of adjuvants, such as alum for stimulating optima responses. Adjuvants are often associated with toxicity and have been shown to nonspecifically stimulate the immune system; thus inducing antibodies of specificities that may be undesirable.

Another disadvantage associated with T-dependent antigens is that very small proteins such as peptides, are rarely immunogenic, even when administered with adjuvants. This is especially unfortunate because many synthetic peptides are available today that have been carefully synthesized to

represent the primary antigenic determinants of various pathogens, and would otherwise make very specific and highly effective vaccines.

In contrast, T-independent antigens, such as polysaccharides, are able to stimulate immune responses in the absence of adjuvants. Unfortunately, however, such T-independent antigens cannot stimulate high level or prolonged antibody responses. An even greater disadvantage is their inability to stimulate an immature or B cell defective immune system (Mond J.J., Immunological Reviews 64:99, 1982) Mosier DE, et al., J. Immunol. 119:1874, 1977). Thus, the immune response to both T-independent and T-dependent antigens is not satisfactory for many applications.

With respect to T-independent antigens, it is critical to provide protective immunity against such antigens to children, especially against polysaccharides such as H. influenzae and S. pneumoniae. With respect to T-dependent antigens, it is critical to develop vaccines based on synthetic peptides that represent the primary antigenic determinants of various pathogens.

One approach to enhance the immune response to T-independent antigens involves conjugating polysaccharides such H. influenzae PRP (Cruse J.M., Lewis R.E. Jr. ed., Conjugate vaccines in Contributions to Microbiology and Immunology, vol. 10, 1989) or oligosaccharide antigens (Anderson PW, et al., J. Immunol. 142:2464, 1989) to a single T-dependent antigen such as tetanus or diphtheria toxoid. Recruitment of T cell help in this way has been shown to provide enhanced immunity to many infants that have been immunized. Unfortunately, only low level antibody titers are elicited, and

only some infants respond to initial immunizations. Thus, several immunizationa are required and protective immunity is often delayed for months. Moreover, multiple visits to receive immunizations may also be difficult for families that live distant from medical facilities (especially in underdeveloped countries). Finally, babies less than 2 months of age may mount little or no antibody response even after repeated immunization.

One possible approach to overcoming these problems is to homogeneously disperse the antigen of interest within the polymeric matrix of appropriately sized biodegradable-biocompatible microspheres that are specifically taken up by GALT. Eldridge et al. have used a murine model to show that orally-administered 1-10 micrometer microspheres consisting of polymerized lactide and glycolide, (the same materials used in resorable sutures), were readily taken up into Peyer's patches, and the 1-5 micrometer size were rapidly phagocytized by macrophages. Microspheres that were 5-10 micrometers (microns) remained in the Peyer's patch for up to 35 days, whereas those less than 5 micrometer disseminated to the mesenteric lymph node (MLN) and spleen within migrating MAC-1+ cells. Moreover, the levels of specific serum and secretory antibody to staphylococcal enterotoxin B toxoid and inactivated influenza A virus were enhanced and remained elevated longer in animals which were immunized orally with microencapsulated antigen as compared to animals which received equal doses of non-encapsulated antigen. These data indicate that microencapsulation of an antigen given orally may enhance the mucosal immune response against enteric pathogens. AF/R1 pili mediate the species-specific binding of E. coli RDEC-1

with mucosal glycoproteins in the small intestine of rabbits and are therefore an important virulence factor. Although AF/R1 pili are not essential for E. coli RDEC-1 to produce enteropathogenic disease, expression of AF/R1 to produce enteropathogenic disease, expression of AF/R1 promotes a more severe disease. Anti-AF/R1 antibodies have been shown to inhibit the attachment of RDEC-1 to the intestinal mucosa and prevent RDEC-1 disease in rabbits. The amino acid sequence of the AF/R1 pilin subunit has recently been determined, but specific antigenic determinants within AF/R1 have not been identified.

In the current study we have used these theortical criteria to predict probable T or B cell epitopes from the amino acid sequence of AF/R1. Four different 16 amino acid peptides that include the predicted epitopes have been synthesized: AF/R1 40-55 as a B cell epitope, 79-94 as a T cell epitope, 108-123 as a T and B cell epitope, and AF/R1 40-47/79-86 as a hybrid of the first eight amino acids from the predicted B cell epitope and the T cell epitope. We have used these peptides as well as the native protein to stimulate the in vitro proliferation of lymphocytes taken from the Peyer's patch, MLN, and spleen of rabbits which have received introduodenal priming with microencapsulated or non-encapsulated AF/R1. Our results demonstrate the microencapsulation of AF/R1 potentiates the cellular immune response at the level of the Peyer's patch, thus enhancing in vitro lymphocyte proliferation to both the native protein and its linear peptide antigens. CFA/I pili, rigid thread-like structures which are composed of repeating pilin subunits of 147 amino acid found on serogroups 015, 025, 078, and 0128 of enterotoxigenic E. coli (ETEC) (1-4,

18). CFA/I promotes mannose resistant attachment to human brush borders (5); therefore, a vaccine that established immunity against this protein may prevent the attachment to host tissues and subsequent disease. In addition, because the CFA/I subunit shares N-terminal amino acid sequence homology with CS1, CFA/II (CS2) and CFA/IV (CS4) (4), a subunit vaccine which contained epitopes from this area of the molecule may protect against infection with various ETEC.

Until recently, experiments to identify these epitopes were time consuming and costly; however, technology is now available which allows one to simultaneously identify all the T cell and B cell epitopes in the protein of interest. Multiple Peptide synthesis (Pepscan) is a technique for the simultaneous synthesis of hundreds of peptides on polyethylene rods (6). We have used this method to synthesize all the 140 possible overlapping actapeptides of the CFA/I protein. The peptides, still on the rods, can be used directly in ELISA assays to map B call epitopes (6. 12-14). We have also synthesized all the 138 possible overlapping decapeptides of the CFA/I protein. For analysis of T cell epitopes, these peptides can be cleaved from the rods and used in proliferation assays (15). Thus this technology allows efficient mapping and localization of both B cell and T cell epitopes to a resolution of a single amino acid (16). These studies were designed to identify antigenic epitopes of ETEC which may be employed in the construction of an effective subunit vaccine.

CFA/I pili consist of repeating pilin protein subunits found on several serogroups of enterotoxigenic E coli (ETEC) which promote attachment to

human intestinal mucosa. We wished to identify areas within the CFA/I molecule that contain immunodominant T cell epitopes that are capable of stimulating the cell-mediated portion of the immune response in primates as well as immunodominant B cell epitopes. To do this, we (a) resolved the discrepancy in the literature on the complete amino acid sequence of CFA/I, (b) immunized three Rhesus monkeys with multiple i.m. injections of purified CFA/I subunit in Freund's adjuvant, (c) synthesized 138 overlapping decapeptides which represented the entire CFA/I protein using the Pepscan technique (Cambridge Research Biochemicals), (d) tested each of the peptides for their ability to stimulate the spleen cells from the immunized monkeys in a proliferative assay (e) synthesized 140 overlapping octapeptides which represented the entire CFA/I protein, and (f) tested serum from each monkey for its ability to recognize the octapeptides in a modified ELISA assay. A total of 39 different CFA/I decapeptides supported a significant proliferative response with the majority of the responses occurring within distinct regions of the protein (peptides beginning with residues 8-40, 70-80, and 126-137). Nineteen of the responsive peptides contained a serine residue at positions 2, 3, or 4 in the peptide, and a nine contained a serine specifically at position 3. Most were predicted to be configured as an alpha holix and have a high amphipathic index. Eight B cell epitopes were identified at positions 3-11, 11-21, 22-29, 32-40, 38-45, 66-74, 93-101, and 124-136. The epitope at position 11-21 was strongly recognized by all three individual monkeys, while the epitopes at 93-101, 124-136, 66-74, and 22-29 were recognized by two of the three monkeys.

Recent advances in the understanding of B cell and T cell epitopes have improved the ability to select probably linear epitopes from the amino acid sequence using theoretical criteria. B cell epitopes are often composed of a string of hydrophilic amino acids with a high flexibility index and a high probability of turns within the peptide structure. Prediction of T cell epitopes are based on the Rothbard method which identifies common sequence patterns that are common to known T cell epitopes or the method of Berzofsky and others which uses a correlation between algorithms predicting amphipathic helices and T cell epitopes.

V. SUMMARY OF THE INVENTION

This invention relates to active core materials such as biologically active agent(s), drug(s), or substance(s) encapsulated within a biodegradable-biocompatable polymeric matrix. In view of the enormous scope of this invention it will be presented herein as Phases I, II, and III. Phase I illustrates the encapsulation of antibiotics within a biodegradable-biocompatable polymeric matrix for the prevention and treatment of wound infections. Phase II illustrates the encapsulation of antigens (including [illegible] and more specifically, oral-intestinal vaccine antigens) within a biodegradable-biocompatable polymeric matrix against diseases such as those caused by enteropathogenic organism. Phase III illustrates the use of a biodegradable-biocompatible polymeric matrix for burst-free programmable sustained release of biologically active agents, inclusive of peptides, over a period of up to 100 days in an aqueous physiological environment.

Controlled drug delivery from a biodegradable-biocompatable matrix offers profound advantages over conventional drug/antigen dosing. Drugs/antigens can be used more effectively and efficiently, less drug/antigen is required for optimal therapeutic effect and, in the case of drugs, toxic side effects can be significantly, reduced or essentially eliminated through drug targeting. The stability of some drugs/antigens can be improved allowing for a longer shelf-life, and drugs/antigens with a short half-life can be protected within the matrix from destruction, thereby ensuring sustained release of active agent over time. The benefit of a continuous sustained release of drug/antigen is beneficial because drug levels can be maintained within a constant therapeutic range and antigen can be presented either continuously or in a pulsatile mode as required to stimulate the optimal immune response. All of this can be accomplished with a single dose of encapsulated drug/antigen.

This invention contemplates, but is not limited to, medically acceptable methods for the effective local delivery of biologically active agents that, of themselves, are directly (e.g. drugs, such as antibiotics) or indirectly (e.g. vaccine antigens) therapeutic or prophylactic. It also includes drugs/agents that elicit/modulate natural biological activity.

Wounds characterized by the presence of infection, devitalized tissue, and foreign-body contaminants have high infection rates and are difficult to treat. This invention describes antibiotic formulation encapsulated within microspheres of a biodegradable-biocompatable polymer that, when applied locally to contaminated or infected wounds, provides immediate, direct, and sustained (over a period up to 100 days), high concentrations of antibiotic in

the wound site (soft tissue and bone). By encapsulating antibiotics and applying them directly, one can achieve a significant reduction in nonspecific binding of the drug to body proteins, a phenomena commonly observed following conventional systemic administration of free drugs. Thus, less drug is required, higher concentrations are maintained at the site of need, and efficacy is enhanced. This approach provides superior treatment over conventional systemic administration of antibiotics for wound infections because higher bacteriocidal concentrations can be achieved and maintained in the wound environment. Higher concentrations kill more bacteria. Applicants' invention for this application is described in Phase I. Furthermore, applicants reasoned that a protective mucosal immune response might be best initiated by introduction of an antigen at the mucosal surface, because unprotected protein antigens delivered in a free form may be degraded or may complex with secretory IgA in the intestinal lumen precluding entry and subsequent processing in local immune cells. The formulation of microspheres containing antigen small enough in size to be phagocytized locally in the gut was envisioned as being able to induce an elevated localized immune response. Applicants' invention for this application is described in Phase II. In summary, applicants propose using several methods for the local application of drugs including: 1) the direct application of the encapsulated drug to a surgical/traumatized area, 2) oral delivery that provides either local deposition of microencapsulated antigen/drugs at mucosal membranes or transport across these membranes to provide local adherence of microencapsulated drugs/antigen to mucosal membranes to provide sustained

release of drug/antigen into soft tissue or a body cavity, and/or 3) sustained intercellular or extracellular drug/antigen release following subcutaneous injection.

In those instances where antibiotics are administered locally, applicants have found that the controlled release of the antibiotic from within a biodegradable-biocompatable polymeric matrix within 14 days to about 4 weeks without significant drug trailing is especially useful. However, if desired, the release of a biologically active agent from a polymeric matrix comprised of an active agent and a blend of uncapped and end-capped biodegradable poly DL(lactide-co-glycolide), can be controlled over a period of 1 to about 100 days without significant drug dumping or trailing. Such novel biocompatible-biodegradable microspheres developed with a burst-free programmable sustained release of biologically active agents, inclusive of polypeptides, are described in applicants' U.S. Patent Application Serial No. 08/590,973 filed January 24, 1996.

When antibiotics are administered systemically in the conventional manner, or locally as contemplated by the applicants, the immune response to the antibiotic and the potential for hypersensitivity and/or anaphylactoid response (especially to beta-lactam antibiotics such as penicillins/ampicillin) is a clinical concern. In early studies the inventors observed a specific IgG response to ampicillin as it was released from the microencapsulated formulation (illustrated in the histogram, Figure 1 and 2). This response is reminiscent of antibody elicited by vaccine antigens in conventional vaccines. The response to vaccine antigens is known to be accentuated by the use of an

adjuvant such as alum. Alum is a crude, less adaptable delivery vehicle than its counterpart, the biodegradable-biocompatable poly DL(lactide-co-glycolide), of this invention - the polymeric matrix. This knowledge stimulated additional studies relevant to the effects of sustain release of agents on the immune response.

There are, in general, two forms of localized delivery which can be achieved with PLGA microspheres-delivery which is localized to individual cells of the body (intracellular delivery); and delivery which is localized to tissues within a specific region of the body (localized extracellular delivery).

Applicants have prepared antibiotic and hepatitis vaccine formulations which functioned by delivering localized extracellular doses of their active agents. This was achieved by using relatively large microspheres which served as a depot for the drug or antigen. Their large size 40-100 microns in diameter precluded their being phagocytized or diffusing throughout the intercellular fluid compartments of the body. Their drug agent loads were thus released within their immediate vicinity which resulted in the generation of very high local concentrations of antibiotic or the release of sufficiently high concentrations of free antigen to induce an immune response.

The large-diameter antibiotic bearing microspheres were originally developed by applicants primarily for topical application on exposed debrided tissues of combat wounds. However, an inherent property exhibited by the antibiotics when topically applied to a wound site is the generation of measurable levels of immune response. This concept of local delivery by

topical application of microspheres to tissue to achieve localized concentrations of therapeutic agents was subsequently applied to the development of an oral vaccine for protection against traveler's diarrhea caused by E. coli. Vaccine antigen was encapsulated into microspheres whose diameters were predominantly in the 5-10 micron size range based on an understanding that microspheres of this size would not readily be either phagocytized or transported across the gut wall into the body. Ingestion of these microspheres thus constituted a localized delivery achieved by topical application of the spheres to the wall tissue of the gut. This topical application resulted in the localized trapping of a small percentage of these sphere into the Peyer's patches where the spheres proceeded to release their antigen in a localized fashion to immune cells located within the intestinal Patches.

The concept of localized sustained local delivery has been further extended to the delivery of analgesics and anesthetics to exposed dental pulp to control pain and inflammatory responses. Again, the PLGA microsphere used for this type of delivery are relatively large (40-100 um in diameter) and serve as a topical depot for localized extracellular release of the drug.

Consistent with their understanding of the inherent immunogenic properties exhibited by active core materials <u>in vivo</u>, applicants have moved on to other non-topical application methods of using their microsphere delivery system. Some of these center on the use of small diameter microspheres ranging from sub micron to under 5 microns in diameter. These spheres allow intracellular targeting of drug or antigen. They also allow for transmucosal delivery of drugs or antigens. The concept of localized delivery in these

instances refers to the localized delivery of drug or agent within individual target cells of the body regardless of their location or distribution within the body. This approach is useful in development of antitubercular, antimalarial, antiviral, and antichlamydial formulations against intracellular parasites. It is also useful for the development of vaccines against intracellular parasites and for direct delivery of agents to presenting cells of the immune system.

Another nontopical application method of using PLGA microspheres resides in their usefulness as injectable depots for drugs intended for either localized or systemic delivery. Typically larger diameter microspheres are used for depots as these are less likely to diffuse away. The local or systemic nature of these delivery systems is, in part a function of the release rate of the drug from the depot and the diffusional and solubility characteristics of the drug being released. Cancer chemotherapeutics, systemic antibiotics, delivery of antibiotics to infected bone are potential application of this system. Additional this non-topical systemic depot application can be extended to the iv injection of cancer-agent laden microspheres to embolize and destroy a malignant tumor. Additionally, the PLGA microspheres can be used as a carrier to deliversubstances useful for the in modification of cells or genes in bioengineering or genetic procedures.

Interest in the concept that antigens encapsulated within a biodegradable-biocompatible polymeric matrix could be formulated as a vaccine with superior efficacy over conventional vaccines, originated from the inventors' own observations that the drug, ampicillin, when sustain released from poly DL(lactide-co-glycolide) elicited antibody production. In these

studies, the applicants were able to measure specific IgG antibodies to free ampicillin and to ampicillin released from microencapsulated ampicillin formulations in the sera of mice previously "treated" with the ampicillin formulations using ELISA. Numerous other studies also document the ability of beta-lactam antibiotic to elicit antibody. Selected, more recent studies whose findings are consistent with earlier discoveries made by applicants when conducting experiements with ampicillin include those by Klein et al. (1993) who detected specific IgG antibodies (IgG and IgG3 subclasses) to the B-lactam ring in patients receiving penicillin therapy, work by Nagakura et al. (1990) which detected specific antibodies to cephalexin, a B-lactam antibiotic in the sera of guinea pigs, and Auci et al. (1993) who detected benzyl penicilloyl specific IgM, IgG IgE, and IgA antibody forming cells in lymphoid cells of mice given benzyl penicilloyl-Keyhole Limpet Hemocyanin. Pharmaceutical compositions of antigens encapsulated with poly DL(lactide-co-glycolide) are described in Phase II. The microspheres of the invention allow for introduction of vaccine antigens to mucosal surfaces in particles that can be subsequently taken up locally by phagocytic cells. Such an approach for both drugs and antigens provides significant advantages in potency and efficacy over conventional systemically administered drugs or vaccines. A partial list of biologically active agents or drugs that will potentially derive significant medical benefits from this delivery system includes: antibacterial agents; peptides; polypeptides; antibacterial peptides; antimycobacterial agents; antimycotic agents; antiviral agents; antiparastic agents;, antifungal; antiyeast agents; hormonal peptides; cardiovascular agents; hormonal

peptides; cardiovascular agents; narcotic antagonists; analgesics; anesthetics; insulins; steroids including HIV therapeutic drugs (including protease inhibitors) and AZT; estrogens; progestins; gastrointestinal therapeutic agents; non-steroidal anti-inflammatory agents; parasympathoimetic agents; psychotherapeutic agents; tranquilizers; decongestants; sedative-hypnotics; non-estrogenic and non-progestional steroids; sympathomimetic agents; vaccines; vitamins; nutrients; anti-migraine drugs; electrolyte replacements; ergot alkaloids; anti-inflammary agents; prostaglandins; cytotoxic drugs; antigens; antibodies; enzymes; growth factors; immunomodulators; pheromones; prodrugs; psychotropic drugs; nicotine; antiblood clotting drugs; appetite suppressants/stimulants and combinations thereof; contraceptive agents include estrogens such as diethyl silbestrol; 17-beta-estradiol; estrone; ethinyl estradiol; mestranol; progestins such as norethindrone; norgestryl; ethynodiol diacetate; lynestrenol; medroxyprogesterone acetate; dimethisterone; megestrol acetate; chlormadinone acetate; norgestimate; norethisterone; ethisterone; melentate; norgestimate; norethisterone; ethisterone; melengestrol; norethynodrel; and spermicidal compounds such as nonyphenoxypolyoxyethylene glycol; benzethonium chloride; chlorindanol; include gastrointestinal therapeutic agents such as aluminum hydroxide; calcium carbonate; magnesium carbonate; sodium carbonate and the like; non-steroidal antifertility agents; parasympathomimetic agents; psychotherapeutic agents; major tranquilizers such as chloropromaquine HCL; clozapine; mesoridazine; metiapine; reserpine; thioridazine; minor tranquilizers such as chlordiazepoxide; diazepam; meprobamate; temazepam and the like; rhinological decongestants;